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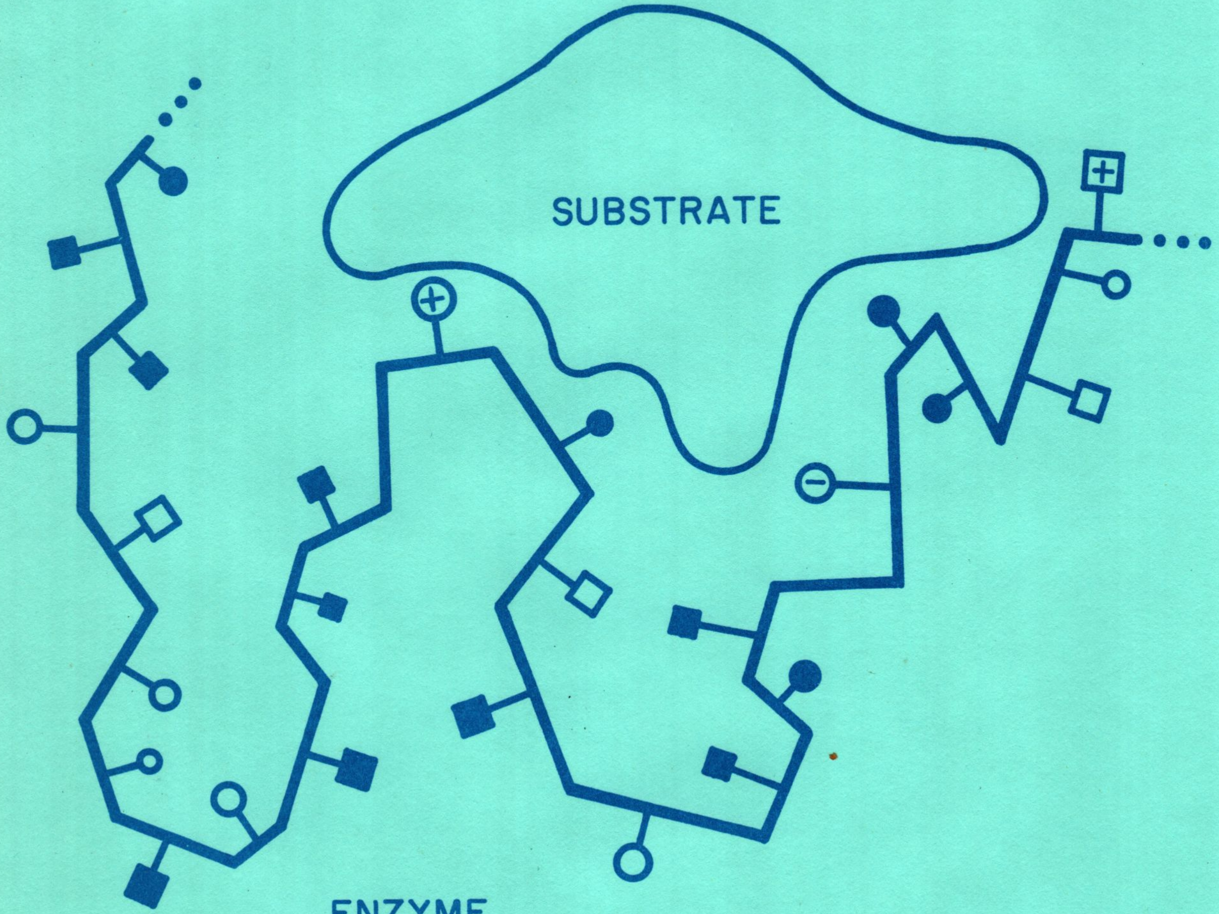
STUDENT MANUAL

PH
THE PH SPECIFICITY OF ENZYMES



HUNTINGTON TWO COMPUTER PROJECT

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14 October 1973

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PH

STUDENT MANUAL

I. INTRODUCTION

Enzymes are pH specific; that is, they work best at certain pH values. For example *pepsin*, the enzyme that digests protein in your stomach, works well at pH values of 1 and 2 but doesn't work at all at pH 9. Why?

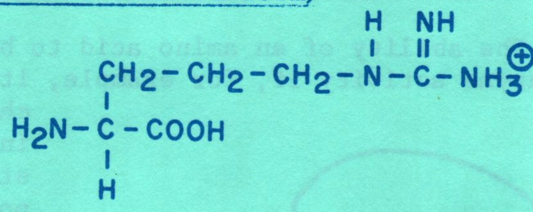
Scientists have not yet agreed on an answer to this question, but there are several theories. The *PH* program allows you to explore one of these theories without having to do the required math.

The theory that the *PH* program is based on holds that pH specificity can be traced to the behavior of ionizable amino acids present at the active site. By *ionizable* we mean that the amino acids can become charged. The *active site* is the particular location on the enzyme where catalysis occurs.

Studies of many different enzymes indicate that there are usually ionized amino acids present at an active site. Amino acids that take a positive (+) charge are called *basic* amino acids, those that take a negative (-) charge acidic amino acids. There are only a few of each kind. Some of these are listed below:

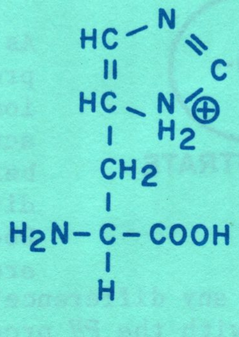
BASIC AMINO ACIDS (shown in ionized form)

ARGININE

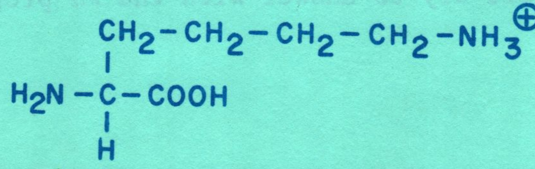


$\mathrm{H_2N-C(H)(COOH)-CH_2-CH_2-CH_2-N(H)-C(=NH)-NH_3^{\oplus}}$

HISTIDINE



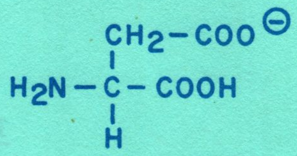
LYSINE



$\mathrm{H_2N-C(H)(COOH)-CH_2-CH_2-CH_2-CH_2-NH_3^{\oplus}}$

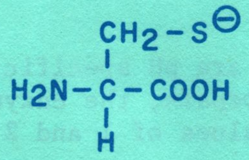
ACIDIC AMINO ACIDS (shown in ionized form)

ASPARTIC ACID



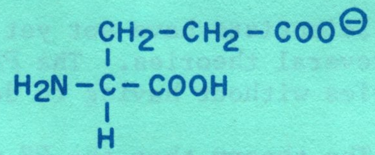
$$\begin{array}{c} \quad\ CH_2-COO^{\ominus} \\ | \\ H_2N-C-COOH \\ | \\ H \end{array}$$

CYSTEINE



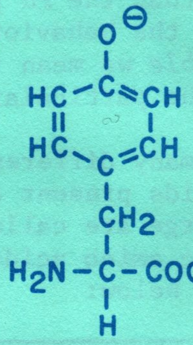
$$\begin{array}{c} \quad CH_2-S^{\ominus} \\ | \\ H_2N-C-COOH \\ | \\ H \end{array}$$

GLUTAMIC ACID

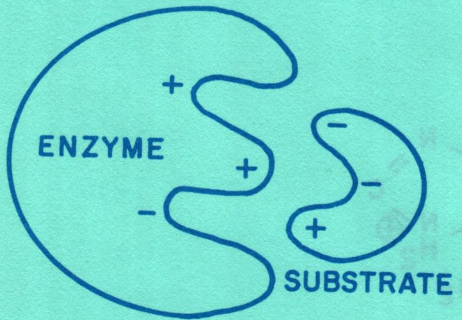


$$\begin{array}{c} \qquad\quad CH_2-CH_2-COO^{\ominus} \\ | \\ H_2N-C-COOH \\ | \\ H \end{array}$$

TYROSINE



The ability of an amino acid to become charged can be very important to enzyme activity if, for example, it is the attraction of opposite charges that is responsible for holding the enzyme's *substrate* (the substance the enzyme works on) in correct position on the surface of the enzyme during catalysis.



As we said before, pH specificity can probably be traced to the behavior of ionizable amino acids present at the active site, the explanation for this being the different pH values at which different amino acids become charged. Does it matter how many amino acids are present at a site? Does the type of amino acid make any difference? These are some of the questions you will try to answer with the *PH* program.

INVESTIGATION #1 *PATTERNS OF ENZYME ACTIVITY*

The *PH* model predicts high enzyme activity when the active site is properly charged and low enzyme activity when there is an improper charge at the active site. As you have already read, the ionizable amino acids become charged *only at certain pH values,* some at high pH only and some at low pH only. Through this investigation you should be able to figure out at what pH each of the seven ionizable amino acids is charged.

Experiment 1

WHAT PATTERN OF ENZYME ACTIVITY
WOULD BE EXPECTED IF AN ENZYME
HAD ONLY ONE CHARGED ACIDIC
AMINO ACID AT ITS ACTIVE SITE?

To answer the question it will be necessary to run *PH* at least once and possibly several times. If you find it necessary to run *PH* more than once, remember that in a controlled experiment only one thing is varied from experiment to experiment, all other variables remaining constant.

The program will first ask you for a series of inputs:

HOW MANY IONIZABLE AMINO ACIDS AT ACTIVE SITE?

The question above gives you the answer to this question. You may want to enter this answer on an INPUT SHEET (see end of this Manual).

The computer will next ask:

AMINO ACID 1 ---- CODE NO.?

These are the code numbers for each of the acids:

1 = aspartic acid
2 = cysteine
3 = glutamic acid
4 = tyrosine
5 = arginine
6 = histidine
7 = lysine

Numbers 1 through 4 are acidic amino acids, while numbers 5 through 7 are basic amino acids.

```
SHOULD IT BE CHARGED (1=YES, 0=NO)?
```

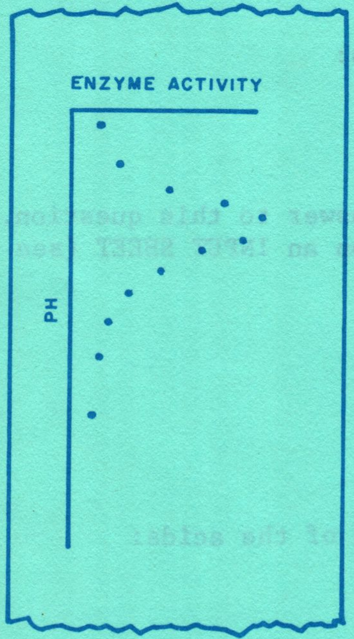
We assume in this experiment that the amino acid must be in the charged condition for enzyme activity.

```
PH RANGE FOR THIS EXPERIMENT:
  LOWER LIMIT?
  UPPER LIMIT?
```

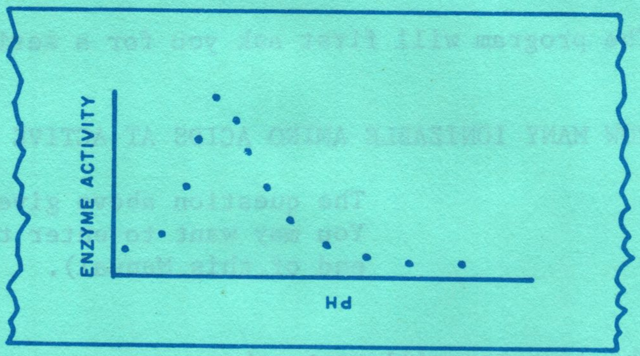
Since we are interested in enzyme activity over the entire pH range, you should select your limits accordingly. (You are limited to the range from 0 to 14.)

REMEMBER: It would be a good idea for you to plan your experiment and record your inputs before you go to the computer. You can use one of the COMPUTER INPUT SHEETS included at the back of this Manual.

When you have finished entering your inputs, the computer will carry out the necessary calculations and then give you the results in graphical form. Enzyme activity will be plotted on the y-axis, and pH on the x-axis. Remember that a computer graph always comes out *sideways*, so you will have to turn it on its side for easier reading.



AS IT COMES OFF THE TELETYPE



TURNED OVER FOR READING

Now, see if you can answer the following question:

OVER WHICH pH RANGE, FROM 0-7 OR 7-14, WOULD AN ENZYME THAT REQUIRES ONE CHARGED ACIDIC AMINO ACID WORK BEST?

Experiment 2 — WHAT ENZYME ACTIVITY PATTERN WOULD BE EXPECTED FOR AN ENZYME THAT REQUIRES ONE CHARGED BASIC AMINO ACID AT THE ACTIVE SITE FOR ENZYME ACTIVITY?

If you did not carry out Experiment 1, please read Experiment 1 instructions first.

Since the only change for Experiment 2 is in the type of ionizable amino acid present, your inputs should differ for *only one* question.

AMINO ACID 1 ---- CODE NO.?

Amino acids coded 5 – 7 are basic amino acids.

Again, be sure to record your inputs on a COMPUTER INPUT SHEET before going to the machine.

At the end of this experiment you should be able to answer the question:

OVER WHICH pH RANGE, 0-7 OR 7-14, SHOULD AN ENZYME THAT REQUIRES ONE CHARGED BASIC AMINO ACID WORK BEST?

Experiment 3 — WHAT PATTERN OF ENZYME ACTIVITY WOULD BE ASSOCIATED WITH AN ENZYME THAT REQUIRES TWO CHARGED AMINO ACIDS AT THE ACTIVE SITE, ONE ACIDIC AND ONE BASIC?

If you did not carry out Experiment 1, be sure to read the instructions starting on page 3 before going on.

For this experiment you will have to enter inputs for the following questions:

- HOW MANY IONIZABLE AMINO ACIDS AT ACTIVE SITE?
- AMINO ACID 1 ---- CODE NO.?

  SHOULD IT BE CHARGED (1=YES, 0=NO)?
- AMINO ACID 2 ---- CODE NO.?

  SHOULD IT BE CHARGED (1=YES, 0=NO)?
- PH RANGE FOR THIS EXPERIMENT?

  LOWER LIMIT?
  UPPER LIMIT?

Since there are many possible combinations of amino acids that can be used in this experiment, it may be wise to do several runs or to compare your results with those of other groups doing the same experiment.

Don't forget to record your inputs on one of the computer INPUT SHEETS (included at the back of this Manual) before you begin.

When you have completed Experiment 3, you should be able to answer the following question:

> *WHAT GENERAL PATTERN OF ACTIVITY CAN BE EXPECTED FOR AN ENZYME WITH TWO IONIZED AMINO ACIDS AT ITS ACTIVE SITE, IF ONE IS ACIDIC AND THE OTHER BASIC?*

FOLLOW-UP QUESTIONS FOR *INVESTIGATION #1*

1) At what pH values (high or low) are the acidic amino acids charged?

2) At what pH values (high or low) are the basic amino acids charged?

3) Pepsin works best only at low pH values of 1 or 2. What type(s) of charged amino acids might be found at its active site?

4) Most enzymes in living cells work best around a pH of 6, 7 or 8 and do not work at all in very high or very low pH's. What kind(s) of amino acids might cause this behavior?

INVESTIGATION #2 *EFFECT ON ENZYME ACTIVITY OF THE NUMBER OF IONIZABLE AMINO ACIDS AT THE ACTIVE SITE.*

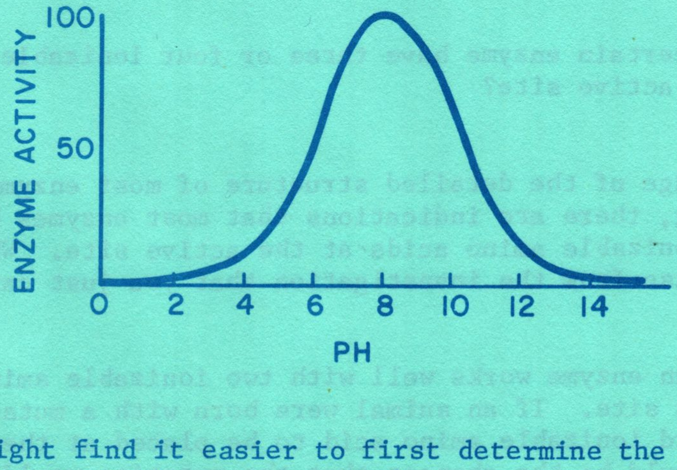
As you may have noticed in *INVESTIGATION #1*, an increase in the number of amino acids often restricts the range of pH in which the enzyme will function. This is often desirable; for instance, we want pepsin to function only in the strongly acidic stomach and not in the slightly alkaline intestine.

This raises the question that if two amino acids at the active site (one basic and the other acidic) can produce a restricted activity range, why should any enzyme have more than two ionizable amino acids at its active site? The answer to this question lies in the possibility of the ionizable amino acids doing more than just restricting the pH range over which the enzyme functions; they may also be taking part in the catalysis of the substrate. Therefore, if an enzyme required more than two types of ionizable amino acids to carry out catalysis, there would be more than two present.

This investigation is designed to allow you to explore the consequences of adding one or two more charged amino acids at the active site. You may follow the outline of experiments offered here, or (if your instructor allows) experiment on your own.

Experiment 1 IS THERE MORE THAN ONE COMBINATION OF TWO AMINO ACIDS THAT WILL PRODUCE A MAXIMUM ACTIVITY IN THE RANGE OF pH 7?

By trying different combinations of two amino acids (they need not be different), you should be able to find a combination that produces a pattern of maximum enzyme activity at pH 7 with a sharp falling off of activity to either side (see sample graph below).



(HINT: You might find it easier to first determine the individual properties of the amino acids.)

Experiment 2 WHAT IS THE EFFECT OF ADDING A THIRD AMINO ACID AT THE ACTIVE SITE?

Once you have completed Experiment 1, it is possible to extend that line of inquiry to see the effect of adding a third ionizable amino acid at the active site. What is the effect of adding an amino acid already present as opposed to an amino acid not currently at the active site?

Question: The addition of which amino acid had the least effect on activity?

Experiment 3 WHAT IS THE EFFECT OF ADDING A FOURTH AMINO ACID AT THE ACTIVE SITE, ASSUMING THAT ACTIVITY IS MODERATE TO HIGH WITH THREE IONIZABLE AMINO ACIDS PRESENT?

Select the combination from Experiment 2 that resulted in the highest relative activity at pH 7. Find the effect of adding another amino acid at the active site.

Question: If enzyme activity is high with three ionizable amino acids present, will the addition of a fourth amino acid improve the relative activity of the enzyme?

(Optional) Is there another combination of amino acids that will produce higher activity at pH 7?

FOLLOW-UP QUESTIONS FOR *INVESTIGATION #2*

1) All other factors being the same, does the addition of another ionizable amino acid improve the activity of an enzyme?

2) Why might a certain enzyme have three or four ionizable amino acids at its active site?

3) While knowledge of the detailed structure of most enzymes is still lacking, there are indications that most enzymes have only one or two ionizable amino acids at the active site. Why might this make sense from the investigation that you just carried out?

4) Assume that an enzyme works well with two ionizable amino acids at the active site. If an animal were born with a mutation that caused a third ionizable amino acid to be placed at the active site, what would be the chances that the mutation would be passed on? WHY?

INVESTIGATION #3 *INVESTIGATION OF THE ACTIVE SITE OF THE ENZYME ACETYLCHOLINESTERASE*

The enzyme acetylcholinesterase catalyzes the breakdown of the chemical acetylcholine. This reaction is very important for the proper functioning of the nervous system.

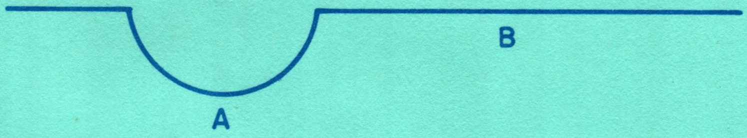
First you'll be given some necessary background on this enzyme and its active site, then you'll be asked to hypothesize which ionizable amino acids might be located at the active site. This question you can attempt to answer using the *PH* program. The method of investigation is totally up to you.

Background - Acetylocholinesterase catalyzes the following reaction in living systems:

Acetylcholine $\longrightarrow$ Acetic Acid + Choline

pH studies of this enzyme show it to have a maximum activity at approximately 8.5. When its activity is plotted against pH, a "bell-shaped curve" results.

Studies of both the enzyme and the substrate indicate the active site may look like the following:



It is thought that there are two ionizable amino acids at the active site. The amino acid at site A in the diagram above is thought to carry a negative (–) charge while the amino acid at site B is thought to remain uncharged when the enzyme is active.

> Your task is to determine which amino acid(s) might be at sites A and B.

REMEMBER: *Only the amino acid at site A is charged. (The amino acid at Site B could be charged initially, but for the period of time when the enzyme is active it does not carry a charge.)*

FOLLOW-UP QUESTIONS FOR *INVESTIGATION #3*

1) Should the amino acid at site A be acidic or basic? WHY?

2) If there were no ionizable amino acid at site B, what would the sketch of the activity curve look like?

3) If there were an uncharged ionizable amino acid at site B, should it be acidic or basic? WHY?

4) What amino acid(s) is probably at site A?

5) What amino acid(s) is probably at site B?

6) (Optional) Are there any other combinations of amino acids (charged or uncharged) that might produce maximum activity at or near pH 8.5?

Name____________________

PH COMPUTER INPUT SHEET

*INVESTIGATION #*____ *Experiment #*____

	RUN 1	RUN 2	RUN 3	RUN 4
NO. IONIZABLE AMINO ACIDS	______	______	______	______
AMINO ACID 1 CODE NO.	______	______	______	______
CHARGED (1=YES, 0=NO)	______	______	______	______
AMINO ACID 2 CODE NO.*	______	______	______	______
CHARGED (1=YES, 0=NO)	______	______	______	______
AMINO ACID 3 CODE NO.*	______	______	______	______
CHARGED (1=YES, 0=NO)	______	______	______	______
AMINO ACID 4 CODE NO.*	______	______	______	______
CHARGED (1=YES, 0=NO)	______	______	______	______
PH RANGE:				
LOWER LIMIT	______	______	______	______
UPPER LIMIT	______	______	______	______

---

*If you are using fewer than 4 ionizable amino acids, leave an appropriate number of these blank.